

VU Research Portal

Characterization of the specific interaction between sialoadhesin and sialylated *Campylobacter jejuni* lipooligosaccharides.

Heikema, A.P.; Bergman, M.P.; Richards, H.; Crocker, P.R.; Gilbert, M.; Samsom, J.N.; Wamel, W.J.; Endtz, H.Ph.; van Belkum, A.

published in

Infection and Immunity
2010

DOI (link to publisher)

[10.1128/IAI.01273-09](https://doi.org/10.1128/IAI.01273-09)

document version

Publisher's PDF, also known as Version of record

[Link to publication in VU Research Portal](#)

citation for published version (APA)

Heikema, A. P., Bergman, M. P., Richards, H., Crocker, P. R., Gilbert, M., Samsom, J. N., Wamel, W. J., Endtz, H. P., & van Belkum, A. (2010). Characterization of the specific interaction between sialoadhesin and sialylated *Campylobacter jejuni* lipooligosaccharides. *Infection and Immunity*, 78(7), 3237-3246.
<https://doi.org/10.1128/IAI.01273-09>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:

vuresearchportal.ub@vu.nl

Characterization of the Specific Interaction between Sialoadhesin and Sialylated *Campylobacter jejuni* Lipooligosaccharides

Astrid P. Heikema, Mathijs P. Bergman, Hannah Richards,
Paul R. Crocker, Michel Gilbert, Janneke N. Samsom,
Willem J. B. van Wamel, Hubert P. Endtz and Alex van
Belkum

Infect. Immun. 2010, 78(7):3237. DOI: 10.1128/IAI.01273-09.
Published Ahead of Print 26 April 2010.

Updated information and services can be found at:
<http://iai.asm.org/content/78/7/3237>

SUPPLEMENTAL MATERIAL

These include:

<http://iai.asm.org/content/suppl/2010/06/09/78.7.3237.DC1.html>

REFERENCES

This article cites 41 articles, 22 of which can be accessed free
at: <http://iai.asm.org/content/78/7/3237#ref-list-1>

CONTENT ALERTS

Receive: RSS Feeds, eTOCs, free email alerts (when new
articles cite this article), [more»](#)

Information about commercial reprint orders: <http://iai.asm.org/site/misc/reprints.xhtml>
To subscribe to to another ASM Journal go to: <http://journals.asm.org/site/subscriptions/>

Characterization of the Specific Interaction between Sialoadhesin and Sialylated *Campylobacter jejuni* Lipooligosaccharides[†]

Astrid P. Heikema,^{1*} Mathijs P. Bergman,³ Hannah Richards,⁴ Paul R. Crocker,⁴ Michel Gilbert,⁵ Janneke N. Samsom,² Willem J. B. van Wamel,¹ Hubert P. Endtz,^{1,6} and Alex van Belkum¹

Department of Medical Microbiology and Infectious Diseases¹ and Laboratory of Pediatric Gastroenterology,² Erasmus MC, University Medical Centre Rotterdam, Rotterdam, Netherlands; Department of Molecular Microbiology, Faculty of Earth and Life Sciences, Vrije Universiteit Amsterdam, Amsterdam, Netherlands³; Department of Cell Biology and Immunology, College of Life Sciences, University of Dundee, Dundee, United Kingdom⁴; Institute for Biological Sciences, Ottawa, Ontario, Canada⁵; and International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh⁶

Received 12 November 2009/Returned for modification 14 December 2009/Accepted 8 April 2010

In *Campylobacter jejuni*-induced Guillain-Barré syndrome (GBS), molecular mimicry between *C. jejuni* lipooligosaccharide (LOS) and host gangliosides leads to the production of cross-reactive antibodies directed against the peripheral nerves of the host. Currently, the presence of surface exposed sialylated LOS in *C. jejuni* is the single known bacterial pathogenesis factor associated with the development of GBS. Using a unique, well-characterized strain collection, we demonstrate that GBS-associated *C. jejuni* strains bind preferentially to sialoadhesin (Sn, Siglec-1, or CD169), a sialic acid receptor found on a subset of macrophages. In addition, using a whole-cell enzyme-linked immunosorbent assay (ELISA), *C. jejuni* strains with sialylated LOS bound exclusively to soluble Sn. Mass spectrometry revealed that binding was sialic acid-linkage specific with a preference for $\alpha(2,3)$ -linked sialic acid attached to the terminal galactose of the LOS chain as seen in the gangliosides GD1a, GM1b, and GM3. This molecular interaction was also related to functional consequences as a GBS-associated *C. jejuni* strain that bound Sn in a whole-cell ELISA adhered to surface-expressed Sn of Sn-transfected CHO cells but was unable to adhere to wild-type CHO cells. Moreover, a sialic acid-negative mutant of the same *C. jejuni* strain was unable to bind Sn-transfected CHO cells. This is the first report of the preferential binding of GBS-associated *C. jejuni* strains to the Sn immune receptor ($P = 0.014$). Moreover, because this binding is dependent on sialylated LOS, the main pathogenic factor in GBS progression, the present findings bring us closer to unraveling the mechanisms that lead to formation of cross-reactive antibodies in GBS disease.

Campylobacter jejuni, a food-borne Gram-negative bacterium, is the major cause of bacterial gastroenteritis worldwide. In addition to enteritis, infection with *C. jejuni* may also lead to a neurological complication called the Guillain-Barré syndrome (GBS). GBS is an autoimmune disease affecting the peripheral nerves. Antibodies raised by the host during an infection with *C. jejuni* possess the capacity to cross-react with structures on human nerve tissue, resulting in neurological complications for the host (38). Further, high titers of anti-ganglioside antibodies are frequently found in the sera of GBS patients (24, 41). Gangliosides are glycosphingolipids with an extracellular sialylated oligosaccharide chain and a ceramide tail that is embedded in the outer leaflet of the plasma membrane. Although predominantly found in the nervous system, gangliosides are present on other cell surfaces as well.

C. jejuni has lipooligosaccharide (LOS) structures on its outer membrane. Biochemical and structural analysis of LOS outer core oligosaccharides has identified sialylated moieties that are structurally similar to several gangliosides (6, 9, 30).

During infection, the structural similarity between *C. jejuni* LOS and human gangliosides, also known as molecular mimicry, facilitates the induction of anti-ganglioside antibodies and the development of GBS (1, 29, 38, 40). The *C. jejuni* genes involved in ganglioside mimicry are located within the LOS biosynthesis locus, a gene cluster that is interchangeable between strains and is genetically highly diverse (18, 19). Therefore, several LOS classes (A through S) have been identified (31). LOS class, gene alterations, mutations, and mechanisms such as phase variation in the LOS locus contribute to structural variations in the ganglioside mimics produced (19). The presence of LOS biosynthesis locus-encoded genes responsible for synthesis, modification, and transfer of sialic acid, found in LOS classes A, B, and C, is crucial in the induction of anti-ganglioside antibodies and hence GBS (20, 36). Sialylated LOS is also involved in other aspects of *C. jejuni* pathogenesis. *C. jejuni* strains expressing sialylated LOS invade human epithelial intestinal cells significantly more frequently than strains expressing nonsialylated LOS (28). However, the receptor for *C. jejuni* attachment to human epithelial intestinal cells is unknown.

Certain *C. jejuni* strains are known to bind to Siglec-7, a member of the sialic acid binding immunoglobulin-like lectin (Siglec) family (8). Siglecs are present on the cell surface of a range of immune-associated cells and are involved in cell to cell interactions and signaling. A subset of the Siglec family, the CD33-related Siglecs, can serve as regulators of the im-

* Corresponding author. Mailing address: Erasmus MC, University Medical Centre Rotterdam, Department of Medical Microbiology and Infectious Diseases, 's Gravendijkwal 230, 3015 CE, Rotterdam, Netherlands. Phone: 31-10-7032176. Fax: 31-10-4703875. E-mail: a.heikema@erasmusmc.nl.

[†] Supplemental material for this article may be found at <http://iai.asm.org/>.

[‡] Published ahead of print on 26 April 2010.

mune system through immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in their cytoplasmic tail (7, 11). In addition, several recently described human Siglecs, Siglecs-14, -15, and -16 can interact with the immunoreceptor tyrosine-based activation motif (ITAM) adaptor, DAP12 and therefore potentially mediate the activation of intracellular signaling (5, 10).

Sialoadhesin (Sn, Siglec-1, or CD169) is a macrophage-restricted Siglec that has been associated with inflammatory and autoimmune diseases. For example, Sn levels are elevated on activated macrophages within the inflamed organs of several inflammatory disorders, including rheumatoid arthritis, experimental autoimmune encephalomyelitis (EAE), and experimental autoimmune uveoretinitis (EAU) (22, 32, 39). This elevated expression may have functional consequences since Sn-deficient mice show a reduced severity of EAE and EAU (25, 39). With a poorly conserved cytoplasmic tail and the absence of tyrosine-based signaling motifs, Sn seems to be more involved in cell-to-cell communication and ligand binding than intracellular immunoregulation. It has been shown that macrophages expressing Sn can bind and internalize sialylated *Neisseria meningitidis* in an Sn- and sialic acid-dependent manner (26). Further, HIV-1 can interact with Sn, probably via a sialic acid residue on gp120, with binding resulting in enhanced infectivity and facilitates transinfections in alpha interferon (IFN- α)-stimulated CD14⁺ monocytes. Furthermore, Sn gene expression is elevated in CD14⁺ monocytes from patients infected with HIV-1 (34).

Each Siglec has a unique specificity for certain sialylated glycans, with Sn preferring sialic acid conjugates with an $\alpha(2,3)$ galactose (gal) linkage (12). This $\alpha(2,3)$ Gal linkage is often found on the LOS of GBS-associated *C. jejuni* strains.

Because of the connection of *C. jejuni* infection with autoimmune disease such as GBS and its clinical variant Miller-Fisher syndrome (MFS), we investigated whether $\alpha(2,3)$ -linked sialic acid residues on the surface of *C. jejuni* strains could interact with Sn and whether this interaction was characteristic for GBS-associated strains.

MATERIALS AND METHODS

Bacterial strains and culture conditions. The bacterial strains utilized in the present study comprise (i) a group of eight Penner serotype reference strains (3, 35) (see Table S1A in the supplemental material), (ii) 29 well-characterized GBS- or MFS-associated *C. jejuni* strains isolated from GBS and MFS patient stool samples (GB13, GB14, GB26, and GB27 were cultured from the diarrheal stools of family members of two GBS patients after a family outbreak of *C. jejuni* enteritis) (4, 21, 27) (see Table S1B in the supplemental material), (iii) 54 age- and sex-matched enteritis-associated *C. jejuni* strains isolated from Dutch patients with active diarrhea, and (iv) a sialic acid transferase (*cstII*) knockout mutant of a GBS-associated strain (GB11 Δ *cstII*) (20).

C. jejuni strains were cultured from -80°C stocks and maintained on Colombia blood agar (BA) plates (Becton Dickinson BV, Alphen aan den Rijn, Netherlands) supplemented with 10 μg of vancomycin/ml in a microaerophilic atmosphere at 37°C . For culture of the GB11 Δ *cstII* mutant strain, chloramphenicol (20 $\mu\text{g}/\text{ml}$) was added to the growth medium. Prior to experimentation, all strains were cultured overnight on BA plates containing vancomycin only. The LOS outer core structures of the GBS-associated strains utilized here have previously been reported (21). LOS was purified by hot phenol-water extraction as previously described (24). For the enteritis-only strains, we used mass spectrometry data as reported (16) to determine whether sialic acid was present in the LOS outer core. PCR screening to verify the LOS class was performed as previously described (20).

Cell culture and preparation of Fc conjugates. Wild-type Chinese hamster ovary cells (CHO-wt), CHO cells expressing Sn domain 1-17 (CHO-Sn), and

CHO cells expressing an Sn mutant with an amino acid substitution in the sialic acid binding pocket at amino acid position 97 (arginine to alanine; CHO-SnR97A) were generated as previously described (13). Cells were maintained in Ham/F-12 medium (PPA Laboratories, Cölbe, Germany), containing penicillin-streptomycin, 2 mM L-glutamine, and 10% fetal calf serum (FCS) and were routinely grown in plastic 75-cm² flasks (Greiner Bio-One, Alphen aan den Rijn, Netherlands) at 37°C in a humidified 5% CO₂-95% air incubator. With respect to Sn-Fc production, CHO cells expressing murine Sn recombinant Fc fusion protein domain 1-3 (Sn-Fc) or its mutant form (SnR97A-Fc) were generated as previously described (37). The cells were cultured in 225-cm² flasks and expanded into roller bottles in glutamine-free Glasgow minimal essential medium (Sigma-Aldrich, Zwijndrecht, Netherlands) containing 100 μM L-methionine sulfoximine (Sigma-Aldrich), glutamine synthetase (GS) supplement (Sigma-Aldrich), penicillin-streptomycin, and 10% dialyzed FCS (Invitrogen, Leek, Netherlands). The FCS concentration was adjusted to 2% once cells covered ca. 80% of the surface of the bottle. When 100% confluence was reached, cells were put on X-VIVO-10 serum-free medium (Lonza, Verviers, Belgium), and medium containing Sn-Fc or SnR97A-Fc was harvested weekly. The concentration of Sn-Fc and SnR97A-Fc produced was determined by using an Fc-specific enzyme-linked immunosorbent assay (ELISA).

Quantification of Fc conjugates. A 96-well Maxisorb ELISA plate (Nunc, Inc., Uden, Netherlands) was coated with 0.01 mg of goat anti-human IgG (Sigma-Aldrich)/ml, followed by incubation for 2 h at room temperature. After washing, wells were blocked with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS). Series of 5-fold dilutions of the Sn-Fc or SnR97A-Fc conjugates were added to the plates, followed by incubation for 2 h at room temperature. Fivefold dilutions of a sample containing a known concentration of Siglec-Fc were used as a reference. After washing, wells were incubated with 1/2,000-diluted peroxidase-conjugated goat anti-human IgG (Sigma-Aldrich). Plates were washed four times with 0.05% Tween 20 in PBS and developed with 100 μl of TMB substrate (3',3',5',5'-tetramethylbenzidine; Sigma-Aldrich)/well. After an appropriate incubation time (5 to 10 min), the reaction was stopped by adding 100 μl of 2 M H₂SO₄/well. The intensity of the signal was measured spectrophotometrically at 450 nm by using a 96-well microplate reader (Bio-Rad, Veenendaal, Netherlands), and the concentrations were determined.

Sialoadhesin-Fc ELISA. With respect to the ganglioside/SnFc ELISA, equal amounts (300 ng/well) of purified bovine brain ganglioside (GA1, GM1, GM2, GM3, GD1a, GD1b, GD3, and GT1b [Sigma-Aldrich]) diluted in ethanol were applied to 96-well Maxisorb ELISA plates (Nunc, Inc.). The plates were then incubated for 2 h at room temperature, allowing the ethanol to evaporate. For the LOS/Sn-Fc ELISA, plates were coated overnight at 37°C using 2 μg of LOS in PBS plus 0.2% trichloroacetic acid per well. After a washing step, the wells were blocked for 2 h at room temperature and for 2 h at 4°C with 1% BSA in PBS (pH 7.8). In the meantime, 1.25 μg of Sn-Fc conjugate/ml was precomplexed with 1/4,000-diluted peroxidase-conjugated anti-human IgG (IgG-PO) (Sigma-Aldrich) in PBS with 0.05% normal goat serum for 1 h at room temperature with shaking. After a washing step, 100 μl of precomplexed Sn-Fc was added/well, and the plates were incubated for 2 h at room temperature. Plates were washed four times with PBS and developed using TMB as previously mentioned.

Finally, for the *C. jejuni*-Sn-Fc ELISA, fresh overnight *C. jejuni* cultures grown on BA plates were harvested and washed, and the optical density at 600 nm (OD₆₀₀) was adjusted to 0.2 in +/-PBS (i.e., PBS containing 2 mM CaCl₂ and 2 mM MgCl₂). After heat inactivation at 56°C for 45 min, 100 μl of each sample/well was added in duplicate to 96-well Maxisorb ELISA plates (Nunc, Inc.), and the open plates were incubated overnight at 37°C in order to allow the fluid to evaporate. After overnight incubation and washing, wells were blocked for 1 h with 1% BSA in PBS at 37°C . In some cases, the bacteria were pretreated with neuraminidase for 16 h using 0.05 U of *Arthrobacter ureafaciens* α -2,3,6,8,9-neuraminidase (Calbiochem, Breda, Netherlands)/ml in PBS-1% BSA. Thereafter, the ELISA protocol previously described for the ganglioside Sn-Fc ELISA was followed with the exception that the *C. jejuni*/Sn-Fc ELISA plates were washed with PBS containing 0.05% Tween 20 to minimize nonspecific binding.

Statistical procedure. For statistical analysis, the strains were divided into either positive or negative groups depending on their Sn binding properties. Strains exhibiting an OD₄₅₀ value higher than 2 times the background signal plus 2 times the standard deviation were classified as positive for Sn binding and strains with a lower OD₄₅₀ signal as a negative. The chi-square test was used to indicate whether a significant difference existed in Sn binding between GBS-associated and enteritis-only strains. We consider $P \leq 0.05$ to be statistically significant.

FITC labeling of *C. jejuni*. Fresh overnight cultured *C. jejuni* was harvested in +/-PBS and incubated for 1 h with 5 μl of fluorescein isothiocyanate (FITC; 100 mg/ml in dimethyl sulfoxide)/ml with shaking. Bacteria were washed in PBS

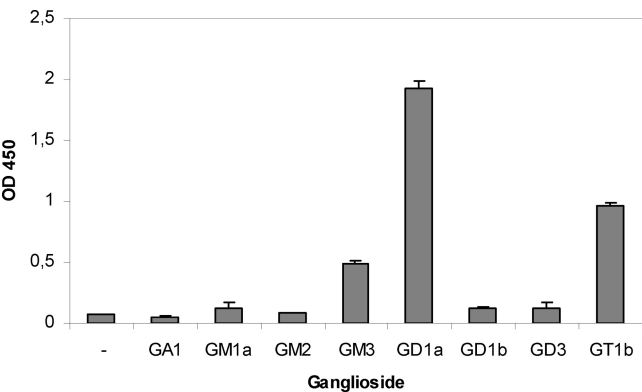


FIG. 1. Sialoadhesin-Fc binding to purified gangliosides. Purified bovine gangliosides dissolved in ethanol were coated on ELISA plates, incubated with Sn-Fc precomplexed with anti-human IgG-PO, and visualized by using TMB. As a control for nonspecific binding of the precomplexed Sn-Fc, ethanol-coated wells were used (–). The data are depicted as means and standard deviations of quadruple measurements. Schematic structures of the gangliosides that were tested are depicted in Fig. 2.

and heat inactivated for 45 min at 56°C, and the OD₆₀₀ was adjusted to 1.0 in +/-PBS.

Binding of *C. jejuni* to Sn-expressing and wild-type CHO cells. CHO-wt, CHO-Sn, and CHO-SnR97A cells were grown to ~80% confluence on glass coverslips and, after being washed with serum-free medium, were incubated for 2 h at 37°C along with various FITC-labeled *C. jejuni* strains in media containing 1% FCS. For this procedure, a bacterium/cell ratio of 100:1 was used. After a washing step, the cells were fixed for 20 min in methanol at –20°C and counterstained using 3 μM propidium iodide (PI). For flow cytometry analysis, semi-confluent CHO cells were harvested from 75-cm² flasks using PBS containing 2

mM EDTA. Cells were incubated for 45 min with FITC-labeled bacteria (bacterium/cell ratio of 100:1) in a 37°C incubator with shaking. After being washed, the cells were analyzed by using a FACSCalibur (Becton Dickinson BV). In control experiments, the cells were incubated for 15 min with 1/10-diluted 3D6, a monoclonal rat anti-mouse antibody raised against Sn, prior to incubation with the bacteria. To confirm Sn expression on CHO-Sn and CHO-SnR97A, 1/100-diluted 3D6 and 1/1,000-diluted Alexa Fluor 633-conjugated goat anti-rat IgG(H+L) secondary antibody (Invitrogen) was used.

RESULTS

Binding of Sn-Fc to purified gangliosides. To validate our batch of Sn-Fc and to extend our knowledge of Sn/ganglioside interactions and affinities using ELISA, we determined the ability of Sn-Fc to bind to a panel of purified bovine brain gangliosides. Gangliosides were coated on ELISA plates and incubated with Sn-Fc precomplexed with anti-human IgG-PO. It has been shown that Sn preferentially binds to α(2,3)-linked sialic acid glycoconjugates with strong affinity for sialic acid residues in the terminal position of a Gal-GalNac-Gal backbone (12). We confirmed these findings with Sn binding properties in a ranking order of GD1a > GT1b > GM3. No Sn binding was observed either when sialic acid was absent (GA1), sialic acid was linked to the inner galactose of (Gal)-GalNac-Gal (GM1a and GM2), or there was an α(2,8)-linkage (GD1b and GD3) (Fig. 1 and 2). In conclusion, the binding efficiency of the Sn variant used here is in agreement with literature data.

Sialic acid-dependent interaction of Sn-Fc with heat-inactivated *C. jejuni* strains and purified LOS. Similar to the ganglioside ELISA described above, we set up a whole-cell bacterial ELISA using eight heat-inactivated *C. jejuni* Penner strains with known ganglioside structures. These included not

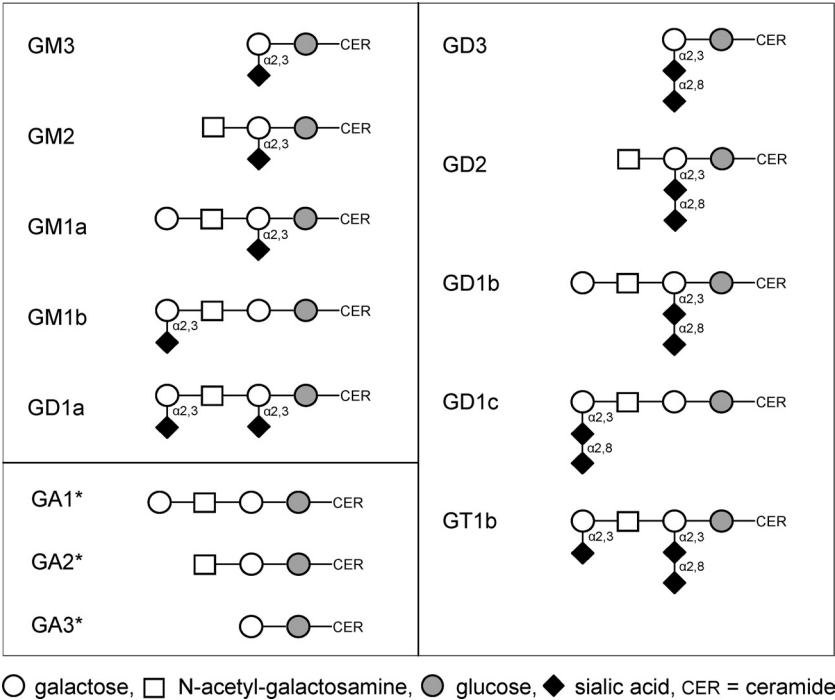


FIG. 2. Schematic representation of the human ganglioside structures relevant to the present study. The galactose-sialic acid linkages are indicated. These structures are mimicked by *C. jejuni* in the outer core LOS. However, instead of the ceramide-bound glucose, the *C. jejuni* LOS has a heptose, followed by an inner sugar core, and *C. jejuni* LOS has a lipid A transmembrane tail instead of a ceramide tail. *, GA1, GA2, and GA3 or asialo-GM1, -GM2, and -GM3 are considered not to be gangliosides.

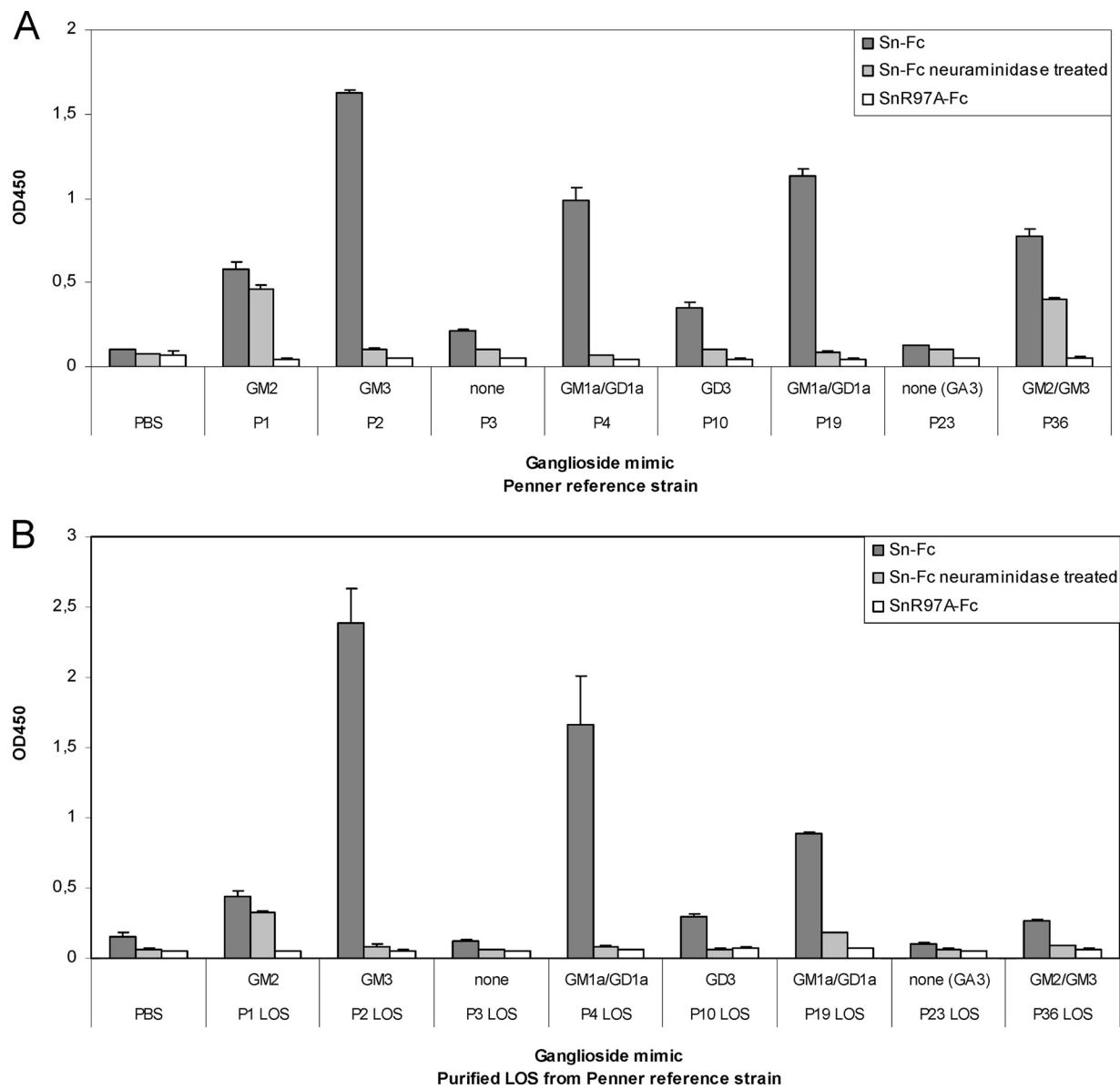


FIG. 3. Heat-inactivated *C. jejuni* Penner strains (A) or purified LOS of the same Penner strains (B) were coated on ELISA plates, incubated with precomplexed Sn conjugates Sn-Fc or SnR97A-Fc, and visualized by using TMB. For binding assays, Penner strains and LOS were either left untreated or were treated with neuraminidase. The bars represent a single experiment which was repeated at least three times, with means and standard deviations of three measurements. Schematic structures of the ganglioside mimics that are expressed by the Penner strains are depicted in Fig. 2.

only Penner strains harboring ganglioside mimics that are the most important in GBS pathology (GM1, GM2, GM3, GD1a, and GD3) but also control strains possessing no ganglioside mimic (none or GA3). Consistent with the ganglioside binding pattern, we found strong Sn binding for strains P2 (GM3), P4 (GM1, GD1a), and P19 (GM1a, GD1a), all having terminal $\alpha(2,3)$ -linked sialic acid residues in the LOS. Sn binding was also observed for P36 (GM2 and GM3). Surprisingly, P1 (GM2) and P10 (GD3) also showed binding to Sn. It is possible that the presence of some undetected GM3 is responsible for this phenomenon. Binding of Sn to strains P3 (none) and P23 (GA3), which lack ganglioside mimics, was found to be low or zero (Fig. 3A). To confirm that these interactions were sialic

acid dependent, we treated the Penner strains with neuraminidase before incubation with the Sn-Fc conjugate. Neuraminidase treatment completely abolished Sn recognition of P2 (GM3), P4 (GM1, GD1a), P10 (GD3), and P19 (GM1, GD1a), showing that Sn binding to these strains is sialic acid specific. Sn binding to P1 (GM2) and P36 (GM2, GM3) was reduced, although not to background levels. Reduction in binding is probably due to the loss of the GM3 mimic. GM2, with an internal sialic acid, is less sensitive to neuraminidase treatment, so residual binding might be because of the presence of this structure.

The ELISA was also performed with the Sn mutant SnR97A-Fc. This mutant has an amino acid substitution

(R97A) in the sialic acid binding pocket and lacks the ability to bind sialic acid conjugates. None of the strains showed binding to SnR97A, ruling out nonspecific binding to other parts of the protein (Fig. 3A).

To date, no ganglioside-like or sialylated structures other than the sialylated LOS have been found on the surface of *C. jejuni* strains. Therefore, Sn binding to *C. jejuni* strains as observed in the whole-cell Sn-Fc ELISA is almost certainly due to the presence of sialylated LOS structures on the bacterial surface. To show that LOS is the ligand for Sn, we also performed Sn-Fc and SnR97A-Fc ELISAs on purified LOS (Fig. 3B), using LOS from the same Penner strains as used in Fig. 3A. Other than some relative differences in signal intensity for P19 and P36, the Sn binding pattern for purified LOS was very similar to the Sn binding pattern for intact bacteria, indicating that the interaction is really LOS specific.

Binding of *C. jejuni* to Sn-expressing CHO cells. When attached to ELISA plate wells, *C. jejuni* and purified LOS from *C. jejuni* were both able to bind to precomplexed soluble Sn. *In vivo*, however, Sn is exposed on cell surfaces. Therefore, to test whether sialic acid-dependent binding to soluble Sn could be reproduced using cell surface-expressed Sn, we used CHO cells stably transfected with mouse Sn cDNA (CHO-Sn). Binding of *C. jejuni* to CHO-Sn cells was compared to that of parental CHO cells (CHO-wt), as well as CHO cells transfected with the Sn mutant R97A (CHO-SnR97A). GB11, a GBS-associated strain, and its *Campylobacter* sialic acid transferase knock-out mutant (GB11 Δ *cstII*) were used. These strains were first tested for Sn binding using the Sn-Fc ELISA and, as expected, GB11 (possessing a mix of GM1a- and GD1a-like structures) bound to Sn, whereas GB11 Δ *cstII* (possessing GA1, GA2, and GA3) did not (Fig. 2 and 4A).

Immunofluorescent staining (Fig. 4B) showed a clear association between GB11 and Sn-expressing CHO cells but no binding of GB11 to CHO-wt or CHO-SnR97A cells. GB11 Δ *cstII* did not bind to any of the CHO cell-lines, indicating that the binding of GB11 to CHO-Sn was actually sialic acid dependent. Flow cytometric analysis of CHO cells incubated with FITC-labeled *C. jejuni* (Fig. 4C) confirmed these findings with a shift in the fluorescent signal observed when GB11 incubated with CHO-Sn was compared to GB11 Δ *cstII* incubated with CHO-Sn. This effect was not observed when GB11 and GB11 Δ *cstII* were incubated with CHO-wt or CHO-SnR97A. Further, upon preincubation of CHO-Sn with 3D6, no difference in binding was observed between GB11 and GB11 Δ *cstII*, indicating that the binding was strictly Sn dependent. Together, these results show that *C. jejuni* with ganglioside mimics in their LOS bind to cell-exposed Sn in a sialic acid-dependent manner.

Sn binding properties of GBS-associated and enteritis-only *C. jejuni* strains. Based on the findings that *C. jejuni* was able to bind Sn in a sialic acid linkage-specific manner, with a preference for terminal α (2,3)-linked sialic acid residues, and the knowledge that this sialic acid linkage is frequently found in the LOS of GBS associated strains, we screened a well-characterized GBS-associated strain collection ($n = 29$), and an age- and sex-matched enteritis control group ($n = 54$) for Sn binding. For this process, a whole-cell Sn-ELISA was used with heat-inactivated *C. jejuni* strains coated onto the ELISA plate. A total of 20 (69%) of the 29 GBS-associated strains

tested were found to be positive for Sn binding in the ELISA (Fig. 5A), including all strains containing GD1a-like LOS (GB2, GB3, GB11, GB18, GB21, GB22, GB28, and GB31). The latter strains always expressed a combination of a GD1a and a GM1a ganglioside mimic. Strains GB13 and GB14, expressing a GM1a-like structure only, produced negative Sn binding results in the ELISA. Therefore, and because the bovine ganglioside GM1a did not show binding in the ganglioside ELISA, the GD1a part of the GD1a/GM1a harboring strains is most likely responsible for Sn binding. Other strains that were positive for Sn binding at least had GM1b- or GM2-like LOS present. Strains MF7 and GB23, also with GM2-like LOS, showed little binding. The difference in binding affinity that was observed for strains with similar LOS structures was probably due to differences in expression rates of the mimics.

Strain GB27 showed very strong Sn binding which is surprising since we previously reported that its LOS outer core is a GA1-like structure (21). GB27 LOS genes are identical to LOS genes of GB26 but earlier sequence analysis had shown that the sialyltransferase gene (*cstII*) was variable in these two strains due to phase variation (i.e., alterations in a hypervariable homopolymeric G tract). GB26 had a 9-G tract in *cstII* that predicted a complete translation product consistent with a sialylated outer core, while GB27 had a 10-G tract that predicted a premature translation stop resulting in no sialyltransferase activity in that strain. Because of the heterogeneity of homopolymeric G tracts, we suggest that the *cstII* gene had a 9-G tract and was turned on in the GB27 sample that was tested for Sn binding. We sequenced the *cstII* gene of four samples of minimally passaged GB27 cultures and indeed found that they contained 9-G tracts (turned on) in the *cstII* gene. We also confirmed the presence of a GM1b mimic in the LOS outer core of these samples by electrophoresis-assisted open-tubular liquid chromatography mass spectrometry (see Table S2 in the supplemental material). Clearly, GB27 is a strain that has the capacity to synthesize a GM1b mimic and to bind strongly to Sn.

The GBS-associated strains were significantly more often positive for Sn binding compared to the enteritis strains ($P = 0.014$) (Table 1). For the enteritis strains, 22 (41%) of 54 strains tested were positive (Fig. 5B). All strains that were positive for Sn binding possessed either a class A, a class B, or a class C LOS gene locus. Strains with such an LOS class contain genes involved in sialic acid synthesis, modification and transfer. The LOS outer core of these enteritis-only strains (except for strain 66) have previously been analyzed by electrophoresis-assisted open-tubular liquid chromatography-electrospray mass spectrometry (16). This analysis confirmed that sialic acid was present in the LOS outer core of the enteritis-only strains that were positive for Sn binding. The mass spectrometry analysis of the enteritis-only strains did not allow determination of the complete LOS outer core structures. However, correlations can be made for the strains that had mass species identical to GBS and MFS strains for which the LOS outer core structures are known (see reference 21). For example, strains 19, 49, 71, 109, and 110 all had the same mass species as GB13 and GB14, which were previously shown to display GM1a mimicry. Therefore, it is reasonable to expect that these strains also possess sialic acid in their LOS outer cores but are still negative for Sn binding. Strains 9, 12, 13, 31,

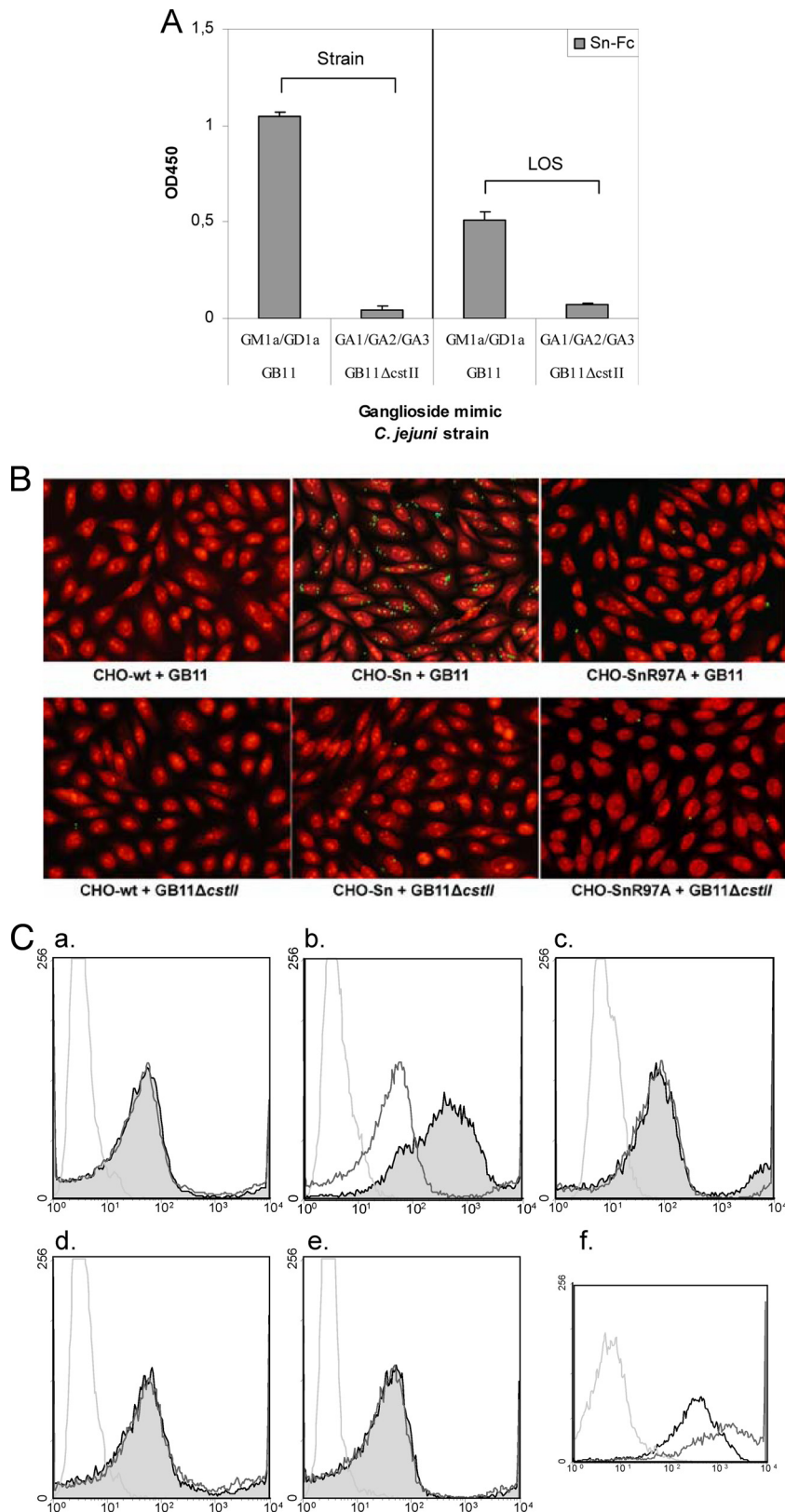


FIG. 4. (A) Sn binding to GBS-associated strain GB11 and the sialic acid-negative mutant GB11ΔcstII. Whole bacteria and purified LOS were tested for Sn binding using ELISA. A schematic representation of the ganglioside mimicking structures expressed by GB11 and GB11ΔcstII can be found in Fig. 2. The structures were determined by mass spectrometry and previously published (20). (B) Immunostaining of wild-type-, Sn-, and SnR97A-expressing CHO cells, incubated with FITC-labeled GB11 or the sialic acid-negative mutant GB11ΔcstII. Cells were counterstained using PI. (C) Flow cytometric analysis of binding between CHO cells and *C. jejuni* strains. Wild-type- and Sn-expressing CHO cells were incubated

and 41 had mass species identical to GB11, which has GM1a/GD1a mimicry. Similar to GB11, these five strains were positive for Sn binding.

DISCUSSION

It has been well established that LOS structures, expressed on the surface of *C. jejuni*, play an important role in development of the postinfectious autoimmune disorders GBS and MFS. Cross-reactive, nerve-damaging antibodies are produced during infection due to molecular mimicry between *C. jejuni* outer core LOS and gangliosides on peripheral nerves. The expression of genes involved in LOS sialylation are a prerequisite for mimicry, since truncated LOS structures without sialic acid show a reduced reactivity with GBS patient serum and fail to induce an anti-ganglioside antibody response in mice (20).

We report here that the sialylated structures on the surface of *C. jejuni* are able to specifically bind to Sn, an immune receptor of the Siglec family that has been linked to various autoimmune diseases. We show that the ligand for Sn is the outer core LOS. Moreover, binding to Sn is sialic acid dependent as neuraminidase-treated strains, as well as a sialic acid transferase knockout strain, failed to bind to Sn. Sn specifically recognizes LOS structures with a terminal $\alpha(2,3)$ -linked sialic acid conjugate as seen in GD1a, GM3, and GM1b. Crucially, upon screening a large panel of pathogenic GBS-associated and nonpathogenic enteritis strains, significantly more GBS-associated strains bound Sn compared to enteritis-only strains ($P = 0.014$).

What is the consequence of Sn binding? It is unclear whether binding to Sn on host cells affects the fate of *C. jejuni*. Sn is expressed on a subset of macrophages, predominantly on macrophages in the subcapsular sinus (SCS) and medulla of the lymph nodes and on metallophilic macrophages of the spleen (14). Lymph node macrophages are involved in capturing and processing of trafficking antigens from the lymph. A recent study (33) showed that SCS lymph node macrophages have limited phagocytic activity; therefore, Sn binding may not be pivotal for intracellular infection by and survival of the bacterium. In contrast, SCS macrophages possess the ability to retain antigens on their cellular surface, which may lead to antigen presentation to follicular dendritic cells (FDC) (23). As such, Sn expression on SCS macrophages may allow capture of soluble ganglioside-mimicking LOS fragments and FDC presentation, leading to B-cell maturation and subsequent antibody production.

Another feature suggesting that Sn is not primarily involved in the initial events of *C. jejuni* invasion comes from our own observations. Although we have shown that *C. jejuni* strains with sialylated LOS invade intestinal epithelial cells better than

nonsialylated strains (28), this effect was found not to be sialic acid linkage dependent. Moreover, intestinal epithelial cells are not known to express Sn. Therefore, factors and/or pattern recognition receptors other than Sn must play a role in *C. jejuni* invasion of the intestinal epithelium.

In the present study we used murine Sn, which has a high degree of protein sequence similarity, up to 79% in the sialic acid binding domain, to human Sn as well as similar $\alpha(2,3)$ -linkage and sialic acid-dependent binding properties (22). Extrapolation to the human situation is therefore plausible.

Our results show that especially strains with a GD1a, GM3, or GM1b ganglioside epitope and only strains with a class A, B, or C LOS were positive for binding to Sn. Only these LOS classes harbor genes involved in LOS sialylation and ganglioside mimicry. Not just GBS-associated strains but also 40.7% of enteritis-only strains were positive for Sn binding. Although these strains possess the Sn binding epitope, infection was not associated with the development of neurological dysfunctions. Therefore, other factors that play a role in macrophage-mediated Sn binding and subsequent processes must contribute to the development of GBS. Single nucleotide polymorphisms (SNPs) that contribute to genetic variations between hosts might alter macrophage function and behavior. Genetic polymorphisms in genes encoding the macrophage-mediators tumor necrosis factor alpha and matrix metalloproteinase-9 have been associated with the more severe forms of GBS (17). Furthermore, coinfection with another bacterial species or virus might direct the immune system toward Sn-mediated autoimmunity. For example, viral infections can induce release of interferons (IFNs), factors that have been shown to upregulate Sn on the surface of monocyte-derived macrophages (15).

Not all GBS-associated strains tested were positive for Sn binding. Strains GB13 (GM1a) and GB14 (GM1a) lack a terminal $\alpha(2,3)$ -linked sialic acid residue that, most likely, is necessary for Sn binding. In fact, these strains were not isolated from a GBS patient but from two family members of a GBS patient during a family related *C. jejuni* enteritis outbreak. Although we classified these strains as GBS-associated because the patient serum reacted with LOS fractions from the *C. jejuni* strains isolated from the family members, a separate uncultured strain might have triggered the development of GBS. Strain GB1 and GB5 with LOS classes C and B harbor genes required for ganglioside mimicry but do not express ganglioside-like epitopes. Sequence analysis of these strains revealed one or more base deletions in LOS-associated genes, resulting in a truncated LOS outer core without sialic acid (21). Anti-GM1 antibodies were detected in the acute-phase serum of the GBS patient from whom strain GB1 was isolated (2). It might be that the base deletions in the LOS of GB1 occurred later in the course of the infection or during laboratory procedures. Strains GB4, GB15, and GB24 lack the genes essential for

with FITC-labeled GB11 or GB11 Δ cstII, with or without preincubation using 3D6 (a monoclonal rat anti-mouse antibody against Sn). Expression of Sn on the CHO cells was confirmed using 3D6 and an Alexa Fluor 633-labeled secondary antibody. (a to c) CHO-wt (a), CHO-Sn (b), and CHO-SnR97A (c), incubated with GB11 and GB11 Δ cstII. (d and e) CHO-wt (d) and CHO-Sn cells (e), preincubated with 3D6 before the addition of GB11 or GB11 Δ cstII. GB11 is depicted as filled gray curves; GB11 Δ cstII is depicted as dark gray open curves. Light gray open curves indicate CHO cells without the addition of bacteria. (f) Expression of Sn (or Sn-R97A) on CHO cells. The light gray line is CHO-wt, the black line is CHO-Sn, and the dark gray line is CHO-SnR97A.

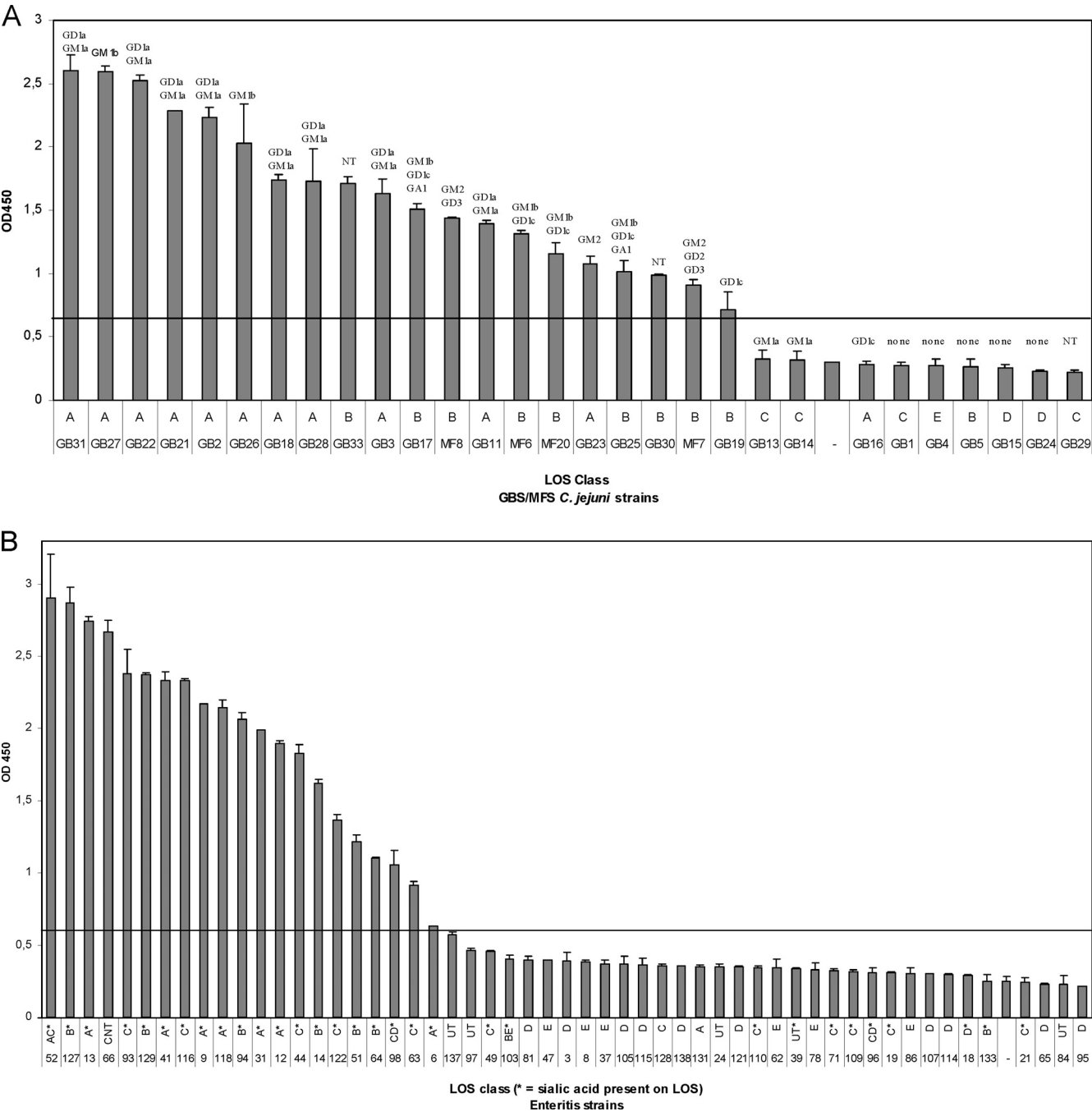


FIG. 5. (A) Sialoadhesin binding to GBS-associated strains. (B) Sialoadhesin binding to enteritis-only strains. Binding of Sn-Fc to GBS-associated strains and enteritis-only strains was evaluated by ELISA. Heat-inactivated bacteria were coated onto plates, incubated with Sn-Fc precomplexed with IgG-PO, and visualized using TMB. The bars represent a single experiment that was repeated at least two times, with the means and standard deviations of two measurements per experiment. -, PBS control for background staining. The black line represents 2 \times (signal plus the standard deviation) the negative control. Strains with OD₄₅₀ values above this line were considered to be positive for Sn binding. UT, untypeable; NT, not tested. Schematic structures of the gangliosides mimics that are expressed by the GBS- and MFS-associated strains are depicted in Fig. 2.

ganglioside mimicry. Although these strains were cultured from the stools of GBS patients, no anti-ganglioside antibodies were detected in the acute-phase sera of these patients (2, 21), suggesting that another pathogenic mechanism than molecular mimicry was involved.

Binding of *C. jejuni* strains to Siglec-7, which has a preference for $\alpha(2,8)$ -linked sialic acid glycans, has already been demonstrated in an earlier study (8). In the same study, however, no binding was found for Sn, not even using LOS purified from strain HS:19 (GM1, GD1a). Although variation in the

TABLE 1. Binding of Sn to GBS or enteritis-related *C. jejuni* isolates^a

Strain association	Count (%) within strains	
	Negative	Positive
GBS	9 (31.0)	20 (69) ^b
Enteritis	32 (59.3)	22 (40.7)

^a Strains were divided into positive or negative for Sn binding characteristics. Strains with OD values higher than 2 times the background signal plus 2 times the standard deviation were classified as positive for Sn binding, and strains with a lower signal were classified as negative for Sn binding.

^b A chi-square test analysis showed that the GBS group of strains exhibited significantly more Sn binding than the enteritis group of strains ($P = 0.014$).

signals between strains was observed in our experiments, all strains possessing GD1a-like LOS we tested were positive for Sn binding. It is unclear, therefore, why no Sn binding was observed with strain HS:19 in this publication (8). We also tested our strains for binding to Siglec-7, and preliminary experiments revealed that especially the MFS-associated strains MF6, MF7, MF8, and MF20 had high affinity for Siglec-7 (data not shown). Although these strains were also positive for Sn binding, the Siglec-7 pathway might generate additional immune responses leading to the specific oculomotor nerve dysfunction seen in MFS. Future studies will focus on this interesting aspect.

In this publication, using various test systems, we demonstrate that *C. jejuni* strains expressing $\alpha(2,3)$ -linked sialylated surface structures are able to bind to Sn. Our finding that GBS-associated strains preferentially bind Sn compared to enteritis strains brings us nearer to an understanding of the mechanisms involved in the formation of cross-reactive antibodies in *C. jejuni*-mediated GBS.

ACKNOWLEDGMENTS

We thank Bart Jacobs and Anne Tio-Gillen, Erasmus MC, Rotterdam, Netherlands, for providing purified bovine brain gangliosides and purified *C. jejuni* LOS. We thank Denis Brochu and Jianjun Li, NRC, Ottawa, Ontario, Canada, for the mass spectrometry analysis of the LOS from *C. jejuni* GB27, and we thank John Hays, Erasmus MC, Rotterdam, Netherlands, for critical reading of the manuscript.

REFERENCES

- Ang, C. W., B. C. Jacobs, and J. D. Laman. 2004. The Guillain-Barre syndrome: a true case of molecular mimicry. *Trends Immunol.* **25**:61–66.
- Ang, C. W., J. D. Laman, H. J. Willison, E. R. Wagner, H. P. Endtz, M. A. de Klerk, A. P. Tio-Gillen, N. van den Braak, B. C. Jacobs, and P. A. van Doorn. 2002. Structure of *Campylobacter jejuni* lipopolysaccharides determines antiganglioside specificity and clinical features of Guillain-Barré and Miller Fisher patients. *Infect. Immun.* **70**:1202–1208.
- Ang, C. W., P. G. Noordzij, M. A. de Klerk, H. P. Endtz, P. A. van Doorn, and J. D. Laman. 2002. Ganglioside mimicry of *Campylobacter jejuni* lipopolysaccharides determines antiganglioside specificity in rabbits. *Infect. Immun.* **70**:5081–5085.
- Ang, C. W., P. A. van Doorn, H. P. Endtz, I. S. J. Merkies, B. C. Jacobs, M. A. de Klerk, R. van Koningsveld, and F. G. A. van der Meché. 2000. A case of Guillain-Barré syndrome following a family outbreak of *Campylobacter jejuni* enteritis. *J. Neuroimmunol.* **111**:229–233.
- Angata, T., Y. Tabuchi, K. Nakamura, and M. Nakamura. 2007. Siglec-15: an immune system Siglec conserved throughout vertebrate evolution. *Glycobiology* **17**:838–846.
- Aspinall, G. O., A. G. McDonald, T. S. Raju, H. Pang, A. P. Moran, and J. L. Penner. 1993. Chemical structures of the core regions of *Campylobacter jejuni* serotypes O:1, O:4, O:23, and O:36 lipopolysaccharides. *Eur. J. Biochem.* **213**:1017–1027.
- Avril, T., H. Floyd, F. Lopez, E. Vivier, and P. R. Crocker. 2004. The membrane-proximal immunoreceptor tyrosine-based inhibitory motif is critical for the inhibitory signaling mediated by Siglecs-7 and -9, CD33-related

- Siglecs expressed on human monocytes and NK cells. *J. Immunol.* **173**:6841–6849.
- Avril, T., E. R. Wagner, H. J. Willison, and P. R. Crocker. 2006. Sialic acid-binding immunoglobulin-like lectin 7 mediates selective recognition of sialylated glycans expressed on *Campylobacter jejuni* lipooligosaccharides. *Infect. Immun.* **74**:4133–4141.
 - Bowes, T., E. R. Wagner, J. Boffey, D. Nicholl, L. Cochrane, M. Benboubetra, J. Conner, K. Furukawa, K. Furukawa, and H. J. Willison. 2002. Tolerance to self gangliosides is the major factor restricting the antibody response to lipopolysaccharide core oligosaccharides in *Campylobacter jejuni* strains associated with Guillain-Barré syndrome. *Infect. Immun.* **70**:5008–5018.
 - Cao, H., U. Lakner, B. de Bono, J. A. Traherne, J. Trowsdale, and A. D. Barrow. 2008. SIGLEC16 encodes a DAP12-associated receptor expressed in macrophages that evolved from its inhibitory counterpart SIGLEC11 and has functional and non-functional alleles in humans. *Eur. J. Immunol.* **38**:2303–2315.
 - Crocker, P. R. 2005. Siglecs in innate immunity. *Curr. Opin. Pharmacol.* **5**:431–437.
 - Crocker, P. R., S. Kelm, C. Dubois, B. Martin, A. S. McWilliam, D. M. Shotton, J. C. Paulson, and S. Gordon. 1991. Purification and properties of sialoadhesin, a sialic acid-binding receptor of murine tissue macrophages. *EMBO J.* **10**:1661–1669.
 - Crocker, P. R., S. Mucklow, V. Bouckson, A. McWilliam, A. C. Willis, S. Gordon, G. Milon, S. Kelm, and P. Bradfield. 1994. Sialoadhesin, a macrophage sialic acid binding receptor for haemopoietic cells with 17 immunoglobulin-like domains. *EMBO J.* **13**:4490–4503.
 - Crocker, P. R., J. C. Paulson, and A. Varki. 2007. Siglecs and their roles in the immune system. *Nat. Rev.* **7**:255–266.
 - Delpitte, P. L., W. Van Breedam, F. Barbe, K. Van Reeth, and H. J. Nauwynck. 2007. IFN- α treatment enhances porcine arterivirus infection of monocytes via upregulation of the porcine arterivirus receptor sialoadhesin. *J. Interferon Cytokine Res.* **27**:757–766.
 - Dziedziatowska, M., X. Liu, A. P. Heikema, R. S. Houlston, A. van Belkum, E. K. Schweda, M. Gilbert, J. C. Richards, and J. Li. 2008. Rapid method for sensitive screening of oligosaccharide epitopes in the lipooligosaccharide from *Campylobacter jejuni* strains isolated from Guillain-Barre syndrome and Miller Fisher syndrome patients. *J. Clin. Microbiol.* **46**:3429–3436.
 - Geleijns, K., M. Emonts, J. D. Laman, W. van Rijs, P. A. van Doorn, P. W. Hermans, and B. C. Jacobs. 2007. Genetic polymorphisms of macrophage-mediators in Guillain-Barre syndrome. *J. Neuroimmunol.* **190**:127–130.
 - Gilbert, M., P. C. R. Godschalk, M.-F. Karwaski, C. W. Ang, A. van Belkum, J. Li, W. W. Wakarchuk, and H. P. Endtz. 2004. Evidence for acquisition of the lipooligosaccharide biosynthesis locus in *Campylobacter jejuni* GB11, a strain isolated from a patient with Guillain-Barré syndrome, by horizontal exchange. *Infect. Immun.* **72**:1162–1165.
 - Gilbert, M., M.-F. Karwaski, S. Bernatchez, N. M. Young, E. Taboada, J. Michniewicz, A.-M. Cunningham, and W. W. Wakarchuk. 2002. The genetic bases for the variation in the lipo-oligosaccharide of the mucosal pathogen, *Campylobacter jejuni*: biosynthesis of sialylated ganglioside mimics in the core oligosaccharide. *J. Biol. Chem.* **277**:327–337.
 - Godschalk, P. C., A. P. Heikema, M. Gilbert, T. Komagamine, C. W. Ang, J. Glerum, D. Brochu, J. Li, N. Yuki, B. C. Jacobs, A. van Belkum, and H. P. Endtz. 2004. The crucial role of *Campylobacter jejuni* genes in anti-ganglioside antibody induction in Guillain-Barre syndrome. *J. Clin. Invest.* **114**:1659–1665.
 - Godschalk, P. C., M. L. Kuijff, J. Li, F. St Michael, C. W. Ang, B. C. Jacobs, M. F. Karwaski, D. Brochu, A. Moterassed, H. P. Endtz, A. van Belkum, and M. Gilbert. 2007. Structural characterization of *Campylobacter jejuni* lipooligosaccharide outer cores associated with Guillain-Barre and Miller Fisher syndromes. *Infect. Immun.* **75**:1245–1254.
 - Hartnell, A., J. Steel, H. Turley, M. Jones, D. G. Jackson, and P. R. Crocker. 2001. Characterization of human sialoadhesin, a sialic acid binding receptor expressed by resident and inflammatory macrophage populations. *Blood* **97**:288–296.
 - Heinen, E., M. Braun, P. G. Coulie, J. Van Snick, M. Moeremans, N. Cormann, C. Kinet-Denoel, and L. J. Simar. 1986. Transfer of immune complexes from lymphocytes to follicular dendritic cells. *Eur. J. Immunol.* **16**:167–172.
 - Jacobs, B. C., M. P. Hazenberg, P. A. van Doorn, H. P. Endtz, and F. G. A. van der Meché. 1997. Cross-reactive antibodies against gangliosides and *Campylobacter jejuni* lipopolysaccharides in patients with Guillain-Barré or Miller Fisher syndrome. *J. Infect. Dis.* **175**:729–733.
 - Jiang, H. R., L. Hwenda, K. Makinen, C. Oetke, P. R. Crocker, and J. V. Forrester. 2006. Sialoadhesin promotes the inflammatory response in experimental autoimmune uveoretinitis. *J. Immunol.* **177**:2258–2264.
 - Jones, C., M. Virji, and P. R. Crocker. 2003. Recognition of sialylated meningococcal lipopolysaccharide by siglecs expressed on myeloid cells leads to enhanced bacterial uptake. *Mol. Microbiol.* **49**:1213–1225.
 - Kuijff, M. L., P. C. Godschalk, M. Gilbert, H. P. Endtz, A. P. Tio-Gillen, C. W. Ang, P. A. van Doorn, and B. C. Jacobs. 2007. Origin of ganglioside complex antibodies in Guillain-Barre syndrome. *J. Neuroimmunol.* **188**:69–73.

28. Louwen, R., A. Heikema, A. van Belkum, A. Ott, M. Gilbert, W. Ang, H. P. Endtz, M. P. Bergman, and E. E. Nieuwenhuis. 2008. The sialylated lipooligosaccharide outer core in *Campylobacter jejuni* is an important determinant for epithelial cell invasion. *Infect. Immun.* **76**:4431–4438.
29. Moran, A. P., and M. M. Prendergast. 1998. Molecular mimicry in *Campylobacter jejuni* lipopolysaccharides and the development of Guillain-Barré syndrome. *J. Infect. Dis.* **178**:1549–1551. (Letter.)
30. Moran, A. P., E. T. Rietschel, T. U. Kosunen, and U. Zahringer. 1991. Chemical characterization of *Campylobacter jejuni* lipopolysaccharides containing *N*-acetylneuraminic acid and 2,3-diamino-2,3-dideoxy-D-glucose. *J. Bacteriol.* **173**:618–626.
31. Parker, C. T., M. Gilbert, N. Yuki, H. P. Endtz, and R. E. Mandrell. 2008. Characterization of lipooligosaccharide-biosynthetic loci of *Campylobacter jejuni* reveals new lipooligosaccharide classes: evidence of mosaic organizations. *J. Bacteriol.* **190**:5681–5689.
32. Perry, V. H., P. R. Crocker, and S. Gordon. 1992. The blood-brain barrier regulates the expression of a macrophage sialic acid-binding receptor on microglia. *J. Cell Sci.* **101**(Pt. 1):201–207.
33. Phan, T. G., I. Grigorova, T. Okada, and J. G. Cyster. 2007. Subcapsular encounter and complement-dependent transport of immune complexes by lymph node B cells. *Nat. Immunol.* **8**:992–1000.
34. Rempel, H., C. Calosing, B. Sun, and L. Pulliam. 2008. Sialoadhesin expressed on IFN-induced monocytes binds HIV-1 and enhances infectivity. *PLoS One* **3**:e1967.
35. Szymanski, C. M., F. S. Michael, H. C. Jarrell, J. Li, M. Gilbert, S. Larocque, E. Vinogradov, and J.-R. Brisson. 2003. Detection of conserved N-linked glycans and phase-variable lipooligosaccharides and capsules from *Campylobacter* cells by mass spectrometry and high resolution magic angle spinning NMR spectroscopy. *J. Biol. Chem.* **278**:24509–24520.
36. van Belkum, A., N. van den Braak, P. Godschalk, W. Ang, B. Jacobs, M. Gilbert, W. Wakarchuk, H. Verbrugh, and H. Endtz. 2001. A *Campylobacter jejuni* gene associated with immune-mediated neuropathy. *Nat. Med.* **7**:752–753.
37. Vinson, M., P. A. van der Merwe, S. Kelm, A. May, E. Y. Jones, and P. R. Crocker. 1996. Characterization of the sialic acid-binding site in sialoadhesin by site-directed mutagenesis. *J. Biol. Chem.* **271**:9267–9272.
38. Willison, H. J., and N. Yuki. 2002. Peripheral neuropathies and anti-glycolipid antibodies. *Brain* **125**:2591–2625.
39. Wu, C., U. Rauch, E. Korpos, J. Song, K. Loser, P. R. Crocker, and L. M. Sorokin. 2009. Sialoadhesin-positive macrophages bind regulatory T cells, negatively controlling their expansion and autoimmune disease progression. *J. Immunol.* **182**:6508–6516.
40. Yuki, N. 1997. Molecular mimicry between gangliosides and lipopolysaccharides of *Campylobacter jejuni* isolated from patients with Guillain-Barré syndrome and Miller Fisher syndrome. *J. Infect. Dis.* **176**:S150–S153.
41. Yuki, N., T. W. Ho, Y. Tagawa, M. Koga, C. Y. Li, K. Hirata, and J. W. Griffin. 1999. Autoantibodies to GM1b and GalNAc-GD1a: relationship to *Campylobacter jejuni* infection and acute motor axonal neuropathy in China. *J. Neurol. Sci.* **164**:134–138.

Editor: B. A. McCormick